PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
A61K 39/395, C12N 15/11, A61K 38/17, C12N 15/63, G01N 33/68, C07K 14/47, A01K 67/027

A2

(11) International Publication Number: **WO 00/21559**

(43) International Publication Date: 20 April 2000 (20.04.00)

(21) International Application Number: PCT/US99/23485

(22) International Filing Date: 8 October 1999 (08.10.99)

(30) Priority Data:
60/103,660 9 October 1998 (09.10.98) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 60/103,660 (CIP)
Filed on 9 October 1998 (09.10.98)

(71) Applicant *(for all designated States except US)*: MUSC FOUNDATION FOR RESEARCH DEVELOPMENT [US/US]; Suite 305, Cannon Park Place, 141 MUSC Complex, Charleston, SC 29425 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: GILKESON, Gary [US/US]; 843 Whispering Marsh Drive, Charleston, SC 29412 (US). WATANABE, Hiroshi [JP/US]; Apartment 6E, 1850 Ashley Crossing Lane, Charleston, SC 29414 (US).

(74) Agents: SPRATT, Gwendolyn, D. et al.; Needle & Rosenberg, P.C., Suite 1200, The Candler Building, 127 Peachtree Street, N.E.; Atlanta, GA 30303–1811 (US).

(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: BLOCKING FACTOR B TO TREAT COMPLEMENT–MEDIATED IMMUNE DISEASE

(57) Abstract

The present invention provides a method of treating or preventing complement–mediated immune disease in a subject, comprising administering to the subject an inhibitor of synthesis or activity of Factor B, whereby the inhibition of synthesis or activity of Factor B treats or prevents the immune disease. Also provided are an antibody to Factor B, an anti–sense oligonucleotide which blocks the mRNA which encodes Factor B and a peptide which competitively binds C3, the receptor for Factor B. The present invention further provides a vector comprising the nucleic acid of this invention and a cell comprising the vector. Also provided is a method of administering to a subject in a pharmaceutically acceptable carrier an antibody, oligonucleotide, peptide and vector of the invention. The present invention further provides a method of determining the predisposition of a subject to complement–mediated immune disease and a method of determining efficacy of treatment in and prognosis of a subject with complement–mediated immune disease. Further, the present invention also provides a mutant transgenic mouse.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

BLOCKING FACTOR B TO TREAT COMPLEMENT-MEDIATED IMMUNE DISEASE

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates to the field of complement-mediated autoimmune diseases. In particular, the present invention relates to the role of the alternative pathway in complement activation and specifically blocking Factor B to treat and prevent complement-mediated autoimmune diseases.

BACKGROUND ART

Activation of the alternative complement pathway is a first line of defense against microorganisms in the absence of specific complement fixing antibody (1-3). Complement Factor B (Bf) is one of the proteins required for activation of the alternative pathway. During activation of the alternative pathway, Bf binds to C3b on a pathogen surface and is cleaved to Ba and Bb by Factor D. Then, properdin binds to the C3bBb complex and stabilizes it. C3bBb complex is a C3 convertase and leads to the formation of more molecules of C3b resulting in activation of additional Bf setting up an amplification loop for activation of C3 (1-3). C3 activation also initiates the cascade of events that leads to immune complex formation. Formation of the C5 convertase C3bBbC3b follows with activation of terminal complement components on the pathogen surface. This process leads to opsonization and direct damage of the pathogen (1-3). Factor B is encoded by a single gene in the MHC Class III gene cluster immediately 3' to the C2 gene; C2 is the functional homologue of Bf in the classical complement pathway (4, 5).

Factor B is produced by hepatocytes (6), phagocytes (7, 8), fibroblasts (9), epithelial cells (10), and endothelial cells (11). Bf is an acute phase reactant; therefore, serum levels of Bf increase during inflammation stimulated by cytokines, growth

factors, and bacterial products (1, 5). Cis-elements responsible for the constitutive and regulated expression of Bf are located in the C2-Bf intergenic region (4). Factor B is not only a component of the alternative complement pathway, but also serves as a B cell growth factor (12, 13), stimulates mononuclear cell cytotoxicity (14, 15), induces macrophage spreading (16), and solubilizes immune complexes (ICs) (17-19). Although Factor B is found deposited in glomeruli in immune complex glomerulonephritis, Factor B has no known role in disease pathogenesis.

In contrast, the classical pathway of the complement system has a known important role in immune complex glomerulonephritis. ICs depositing in the kidney activate the classical pathway, thus eliciting inflammation and tissue destruction (1, 5, 22). Furthermore, components of the classical complement pathway are important in humoral responses to foreign antigens, immune complex clearance, B cell tolerance, and disposition of apoptotic cells (23-27). Genetic deficiency of C3 and therapies directed at blocking C3 activity (e.g. Crry transgenic mice) prevent glomerular inflammation and tissue damage in murine models of induced glomerulonephritis (28-32).

The present data show a profound effect of the Factor B deficient genotype on disease, and indicates that Factor B is indeed an important mediator of complement mediated disease. This is a novel and unexpected finding opening up a new therapeutic approach to the treatment of complement mediated diseases.

Because of the limitations of present treatments for complement-mediated autoimmune diseases, there exists a great need to develop new therapies. The present invention overcomes previous limitations in the art by providing methods of blocking Factor B, antibodies directed against Factor B, peptides which block the receptors of Factor B and an oligonucleotide which inhibits synthesis of Factor B.

SUMMARY OF THE INVENTION

The present invention provides a method of treating or preventing Factor B-

mediated immune disease in a subject, comprising administering to the subject an inhibitor of synthesis or activity of Factor B, whereby the inhibition of synthesis or activity of Factor B treats or prevents the immune disease.

The present invention further provides an anti-sense oligonucleotide capable of hybridizing to the nucleic acid which encodes the protein, Factor B.

The present invention also provides a peptide that mimics the binding properties of Factor B but lacks the ability to activate C3, whereby the binding of the peptide to C3 does not activate C3 and blocks the effects of Factor B on C3, thereby reducing formation and deposition of immune complexes in the subject.

The present invention provides a method of determining the predisposition of a subject to a complement-mediated disease, comprising measuring the serum level and activity of Factor B, and correlating the amount of complement activation with the serum level of factor B.

Further provided is a method of diagnosing a subject as having a Factor B-mediated immune disease, comprising measuring the serum level and activity of Factor B and determining the amount of complement activation.

The present invention also provides a method of determining the efficacy of treatment in and prognosis of a subject with a Factor B-mediated immune disease, comprising measuring the serum level and activity of Factor B and determining the amount of complement activation.

Further provided is a transgenic mouse, comprising a mutation in the Fas gene and a mutation in the gene producing Factor B.

Moreover, the present invention provides a method of determining the efficacy of treatment in and prognosis of a subject with Factor B-mediated immune disease, comprising measuring the serum level and activity of C3, whereby a lower than normal

serum level of C3 indicates a poor prognosis and lack of efficacy of treatment, and whereby a rising serum level of C3 from a lower than normal level to a nearly normal level of C3 indicates efficacy of treatment and an improving prognosis.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Urinary protein excretion by MRL/lpr B-/-, B+/+, and B+/- mice. Data presented are the mean 24 hour protein excretion (mg/mouse/day) of 6 mice +/- standard error in each group. $p<0.01$ B+/- mice versus B-/- mice; $p<0.05$ B+/+ mice versus B-/- mice; $p<0.05$ B+/- mice versus B+/+ mice.

Figure 2. Renal pathology of kidneys from MRL/lpr B-/-, B+/+, and B+/- mice. A) Data presented are the mean renal score +/- standard error in each group. There were no statistically significant differences between B-/- and other groups at the age of 22 weeks ($p=0.13$). At the age of 44 weeks, there was a statistically significant difference between B-/- and B+/+ mice ($p=0.04$). B) H&E stain of a kidney from a MRL/lpr B-/- mouse demonstrating mild proliferation without inflammation or fibrosis (400X). C) PAS stain of a kidney from a B-/- demonstrating minimal glomerular PAS staining (400X). D) H&E stain of a kidney from a MRL/lpr B+/+ mouse with glomerular proliferation and sclerosis, (400x). E) PAS stain of a kidney from a B+/+ mouse demonstrating marked glomerular PAS staining with wire loop lesions. F) PAS stain of a kidney from an MRL/lpr B+/+ mouse demonstrating periarteriolar inflammation and vasculitis as well as glomerular proliferation and hypercellularity (100x).

Figure 3. Serum anti-dsCT DNA antibody levels. Data presented are the mean OD 380 +/- standard error at a 1/100 dilution in each group using dsCT DNA as antigen. $p=0.019$ versus B+/- mice. Titrations of sera are shown in the smaller panels. $p=0.83$ B-/- mice versus B+/+ mice. $p=0.13$ B-/- mice versus B+/+ mice.

Figure 4. IgG3 anti-IgG2a RF levels in MRL/lpr serum. Data shown are the mean 380 nm OD +/- standard error at 1/100 dilution of sera in each group.

Significantly lower levels of IgG3 anti-IgG2a RF levels were observed in serum from B-/- mice at the age of 20 weeks (p=0.003 between B-/- and B+/+, p=0.019 between B-/- and B+/-) and at the age of 24 weeks (+p=0.01 between B-/- and B+/+). The upper panel displays sera titrations. The titer of IgG3 anti-IgG2a RF in the serum of B+/- mice is decreased due to the death of mice by 24 weeks of age that had high titers of IgG3 anti-IgG2a RF.

Figure 5. IgG3 cryoglobulin levels in serum of MRL/lpr mice. Data shown are the mean serum IgG3 cryoglobulin levels (μg/ml) +/- standard error in each group. Cryoglobulin IgG3 levels in serum of B-/- mice were significantly decreased at each time point. $p<0.01$ versus B+/+ mice. The level of IgG3 in the serum of B+/- mice was decreased due to the death of mice with severe disease by 24 weeks of age.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, "a" or "an" or "the" may mean one or more. For example, "a" cell may mean one cell or more than one cell.

The present invention is based, at least in part, on the unexpected and surprising discovery that blocking the synthesis or activity of Factor B significantly reduced the incidence of Factor B-mediated immune disease in a subject. Thus, the present invention provides a new means for preventing and treating Factor B-mediated autoimmune disease in mammals, especially human beings.

The present invention provides a method of treating or preventing complement-mediated immune disease in a subject, comprising administering to the subject an inhibitor of synthesis or activity of Factor B, whereby the inhibition of synthesis or activity of Factor B treats or prevents the immune disease. The immune disease treated in the present invention is mediated at least in part by the activation of Factor B in the alternative complement pathway.

There are many known complement-mediated immune diseases. In particular, Factor B may be instrumental in activating the following diseases: vasculitis, systemic lupus erythematosus, rheumatoid arthritis, myocardial infarction, ischemic/ reperfusion injury, cerebrovascular accident, Alzheimer's disease, transplantation rejection (xeno and allo), all antibody-mediated skin diseases, all antibody-mediated organ-specific diseases (including Type I and Type II diabetes mellitus, thyroiditis, idiopathic thrombocytopenic purpura and hemolytic anemia, and neuropathies), multiple sclerosis, cardiopulmonary bypass injury, membranoproliferative glomerulonephritis, polyarteritis nodosa, Henoch Schonlein purpura, serum sickness, Goodpasture's disease, systemic necrotizing vasculitis, post streptococcal glomerulonephritis, idiopathic pulmonary fibrosis (usual interstitial pneumonitis) and membranous glomerulonephritis.

The present invention provides a method for contacting the subject with an antibody to Factor B, under conditions that permit the binding of the antibody to Factor B in the subject. This binding of the antibody to Factor B reduces the serum level or activity of Factor B, thereby reducing the amount of Factor B available to begin the cascade of events in the alternative complement pathway which enhances activation of the classical complement pathway. With less activation of the classical complement pathway, less inflammation occurs. Also, fewer immune complexes are formed which can be deposited in the organs, thereby decreasing the incidence of disease.

The present invention also provides a method wherein the binding of antibody to Factor B reduces the activation of C3, thereby decreasing complement-induced inflammation. C3 is an enzyme in the complement cascade on which both the classical and alternative pathways act. This antibody may be monoclonal or polyclonal and may be produced by one of ordinary skill in the art.

A purified monoclonal antibody that specifically binds and blocks Factor B is provided. A purified polyclonal antibody that specifically binds and blocks Factor B is also provided. The antibody can specifically bind a unique epitope of Factor B. The term "bind" means the well understood antigen/antibody binding as well as other nonrandom association with an antigen. "Specifically bind" as used herein describes an

antibody or other ligand that does not cross react substantially with any antigen other than the one specified, in this case, Factor B. "Blocks" as used herein means interferes with or prevents the action of."

Factor B or an immunogenic fragment thereof can be injected into an animal in an amount and in intervals sufficient to elicit an immune response. Polyclonal antibodies can be purified directly, or spleen cells from the animal can be fused with an immortal cell line and screened for monoclonal antibody secretion. Thus, purified monospecific polyclonal antibodies that specifically bind the antigen are within the scope of the present invention.

The present invention further provides a composition comprising an antibody to Factor B in a pharmaceutically acceptable carrier. The antibody can be administered to a subject by parenteral administration. As used herein, "parenteral administration" includes intradermal, subcutaneous, intramuscular, intraperitoneal, intravenous and intratracheal routes. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Patent No. 3,610,795, which is incorporated by reference herein. To determine the dosage of the composition to be used in treating Factor B-mediated immune disease, a person of skill in the art would infuse the antibody into a subject after first determining the level of Factor B in the serum of the subject. The dosage in mg/Kg would be based on that amount of antibody necessary to eliminate Factor B from the serum and the activity of Factor B in the serum.

The efficacy of treatment with an anti-Factor B antibody is confirmed in a murine model of Factor B-mediated glomerulonephritis, that is accepted in this field as representative of human glomerulonephritis. A fall in the serum level of Factor B and a rise in the serum levels of C3 and C4, as well as a decrease in the amount of protein excreted in the urine of the subject, indicate that the antibody has bound to Factor B and eliminated the activity of Factor B in the complement system.

The present invention also provides a method of blocking Factor B comprising administering an anti-sense oligonucleotide to block the synthesis of Factor B in a cell of the subject *in vivo*. The anti-sense oligonucleotide interferes with the mRNA necessary for the synthesis of Factor B in the cell and reduces the serum level of Factor B, thereby reducing formation and deposition of immune complexes in the subject. Moreover, the reduction of the serum level of Factor B decreases the level of activation of C3 in the classical complement pathway, thereby reducing the incidence of complement-mediated inflammation and immune disease.

Further, the present invention provides an anti-sense oligonucleotide capable of hybridizing to an mRNA encoded by the Factor B coding sequence (49). Antisense technology is well known in the art and describes a mechanism whereby a nucleic acid comprising a nucleotide sequence which is in a complementary, "antisense" orientation with respect to a coding or "sense" sequence of an endogenous gene is introduced into a cell, whereby a duplex forms between the antisense sequence and its complementary sense sequence. The formation of this duplex results in inactivation of the endogenous gene. Antisense nucleic acids can be produced for any endogenous gene for which the coding sequence has been or can be determined according to well known methods.

Antisense nucleic acids can inhibit gene expression by forming an RNA/RNA duplex between the antisense RNA and the RNA transcribed from a target gene. The precise mechanism by which this duplex formation decreases the production of the protein encoded by the endogenous gene most likely involves binding of complementary regions of the normal sense mRNA and the antisense RNA strand with duplex formation in a manner that blocks RNA processing and translation. Alternative mechanisms include the formation of a triplex between the antisense RNA and duplex DNA or the formation of a DNA-RNA duplex with subsequent degradation of DNA-RNA hybrids by RNAse H. Furthermore, an antisense effect can result from certain DNA-based oligonucleotides via triple-helix formation between the oligomer and double-stranded DNA which results in the repression of gene transcription.

An expression construct including a vector may be used to introduce a nucleic acid encoding the anti-sense oligonucleotide into a cell of the subject. The exact method of introducing the nucleic acid into mammalian cells is, of course, not limited to the use of adenoviral vectors. Other vector systems can be utilized in this invention including the retroviral vector system which can package a recombinant retroviral genome. The recombinant retrovirus can then be used to infect and thereby deliver nucleic acid to the infected cells. Other techniques are widely available for this procedure including the use of adeno-associated viral (AAV) vectors, recombinant herpes virus (HSV), lentiviral vectors, pseudotyped retroviral vectors, and pox virus vectors, such as vaccinia virus vectors. Physical transduction techniques can also be used, such as liposome delivery and receptor-mediated and other endocytosis mechanism. This invention can be used in conjunction with any of these or other commonly used gene transfer methods. The present invention further provides a vector in a composition comprising a pharmaceutically acceptable carrier.

Delivery of the nucleic acid or vector to cells can be via a variety of mechanisms. As one example, delivery can be via a liposome, using commercially available liposome preparations such as LIPOFECTIN, LIPOFECTAMINE (GIBCO-BRL, Inc., Gaithersburg, MD), SUPERFECT (Qiagen, Inc. Hilden, Germany) and TRANSFECTAM (Promega Biotec, Inc., Madison, WI), as well as other liposomes developed according to procedures standard in the art. In addition, the nucleic acid or vector of this invention can be delivered *in vivo* by electroporation, the technology for which is available from Genetronics, Inc. (San Diego, CA) as well as by means of a SONOPORATION machine (ImaRx Pharmaceutical Corp., Tucson, AZ).

The present invention provides a peptide that mimics the binding properties of Factor B but lacks the ability to activate C3, whereby the binding of the peptide to C3 does not activate C3 and blocks the effects of Factor B on C3, thereby reducing formation and deposition of immune complexes in the subject. The peptide of the present invention may bind the same region of C3 to which Factor B normally binds or any other region of C3, whereby the binding of the peptide to C3 blocks the binding of Factor B to C3. Therefore, the peptide can comprise the sequence of amino acids of the

known Factor B sequence that is known to interact with or bind C3. Moreover, a peptide may bind to Factor B in that region of Factor B which binds to C3, thereby blocking Factor B from binding to and activating C3. This sequence can correspond to the region of amino acids of C3 that interact or bind to Factor B.

Moreover, the present invention provides a method for blocking the effect of Factor B on C3, comprising administering to a subject a peptide that mimics the binding properties of Factor B but lacks the ability to activate C3. The binding of the peptide to C3 does not activate C3 and competitively blocks the effects of Factor B on C3, thereby reducing formation and deposition of immune complexes in the subject. Moreover, the peptide binding to C3 blocks activation of C3, thereby decreasing complement-mediated inflammation and immune disease.

The present invention provides a composition comprising a peptide which binds C3 and a pharmaceutically acceptable carrier. The composition can be administered parenterally, including but not limited to intravenously, intramuscularly and intraperitoneally.

Further, the present invention provides an isolated nucleic acid encoding the peptide which binds but does not activate C3. Moreover, a vector comprising the nucleic acid encoding the peptide is provided. The vector can be a commercially available preparation or can be constructed in the laboratory according to methods well known in the art. This vector may be in a cell *in vivo* or *ex vivo*. The present invention also provides a composition comprising the vector and a pharmaceutically acceptable carrier.

Moreover, the present invention provides a method of determining the predisposition of a subject to Factor B-mediated immune disease, comprising measuring the serum level and activity of Factor B and determining the amount of complement activation. A higher than normal level of Factor B indicates complement-mediated immune disease and active inflammation.

The present invention also provides a method of diagnosing a subject as having Factor B-mediated immune disease, comprising measuring the level of anti-DNA antibody in the serum of the subject. A higher than normal level of anti-DNA antibody indicates complement-mediated immune disease and active inflammation. Anti-DNA antibody can be measured in the serum according to the methods taught in the Examples.

Factor B can be measured in the serum by the method taught in the Examples. Serum levels of Factor B can be measured in a population of subjects in whom there is no sign of complement-mediated immune disease to determine a normal range of values. With the same method, a person skilled in the art can measure the serum level of Factor B in a subject with signs of complement-mediated immune disease to determine an abnormal range of values. A higher than normal level of Factor B in a subject would indicate the presence of inflammation and complement-mediated immune disease. Likewise, a cohort can be followed over the long term to identify sub ranges measured within the apparently normal population that predict progression to a diseased state.

The present invention also provides a method of determining the efficacy of treatment in and prognosis of a subject with complement-mediated immune disease, comprising measuring the serum level and activity of Factor B and determining the amount of complement activation. As treatment progresses, the clinician would expect a higher than normal serum level of Factor B to fall to a nearly normal level, if the treatment is effective. Absence of a fall in the serum level of Factor B would indicate lack of efficacy of a therapeutic regimen and continued activation of complement with continuing deposition of immune complexes.

The present invention also provides a transgenic mouse comprising a mutation in the Fas gene and a mutation in the gene producing Factor B. This mutant mouse is the MRL-lpr Factor B knockout mouse.

The present invention provides a method of determining the efficacy of treatment in and prognosis of a subject with Factor B-activated complement-mediated immune disease, comprising measuring the serum level and activity of C3, whereby a lower than

normal serum level of C3 indicates a poor prognosis and lack of efficacy of treatment, and whereby a rising serum level of C3 from a lower than normal level to a nearly normal level of C3 indicates efficacy of treatment and an improving prognosis. The method of measuring the serum level of C3 is taught in the Examples. Moreover, C5A and membrane attack complexes can be measured to monitor the efficacy of treatment in accordance with methods known to a person of skill in the art.

The present invention is more particularly described in the following examples which are intended as illustrative only since numerous modifications and variations therein will be apparent to those skilled in the art.

EXAMPLES

Factor B deficient mice: Factor B deficient mice were derived as previously described (36). B6/129 B+/- mice were intercrossed for 4 generations to yield the B-/- mice used in these studies. A B6/129 intercross B-/- female was bred to an MRL/lpr male. Offspring of this mating were further backcrossed for 3 generations to MRL/lpr mice. B+/- backcross 4 mice were bred to yield the mice used in this study. Eleven B-/-, fourteen B+/+ and ten B+/- mice were included in this study. Mice were maintained in the animal facility of Washington University and the Strom Thurmond Biomedical Research Building at MUSC under specific pathogen free conditions. Routine screening of the mice for murine pathogens was negative.

Genotypic Identification of the mice by PCR: Primers for PCR reactions for Bf were as follows: W1398 (5'; Bf) 5' CCGAAGCATTCCTATCCTCC-3' (SEQ ID NO:1), W1399 (5'; Neo) 5'-CGAATGG GTGACCGCTTCC-3' (SEQ ID NO:2), W1393 (3'; Bf) 5'-GTAGTCTTGTCTGCTTTCTCC-3' (SEQ ID NO:3). DNA was isolated from tail snips (3-4 week old mice) using a QIAamp Tissue Kit (QIAGEN, Santa Clarita, CA). PCR was performed by adding 2ml of DNA (1μg) into a 23μl reaction mixture containing 1.5mM of MgCl2, 6.7 μM each of oligonucleotide mix, 10mM each of dNTP mix, and 0.2ml of Taq Gold. PCR conditions were as follows: 94°C for 4 min.; 28

cycles of 94°C for 1 min., 68°C for 1 min., and 72°C for 2 min; 72°C for 10 min. Following PCR amplification, samples were electrophoresed in a 2% agarose gel and visualized by ethidium bromide staining.

Primers for PCR reactions for Fas were as follows: Fas A (5'-AGGTTAC AAAAGGTCACCC-3') (SEQ ID NO:4), Fas B (5'-GATACGAAGATCCTTTCCTGTG-3') (SEQ ID NO:5), and Fas C (5' CAAACGCAGTCAAATCTGCTC-3') (SEQ ID NO:6). In brief, individual genomic tail DNA was isolated and used in PCR. PCR conditions were as follows: 94°C for 10 min.; 40 cycle of 94°C for 1 min., 50°C for 1 min., and 72°C for 1 min; 72°C for 10 min. Following PCR amplification, samples were electrophoresed in a 2% agarose gel and visualized by ethidium bromide staining.

H2- H2 of the mice was determined by flow cytometric analysis of splenic mononuclear cells. Single cell suspensions were derived and stained with FITC labeled anti-$H2D^k$ (monoclonal antibody KH95) or anti-$H2D^b$ (monoclonal antibody 15-5-5, Pharmingen, San Diego, CA). FITC labeled isotype control antibodies were used to eliminate non-specific staining as a variable. Samples were analyzed on a Becton Dickinson FACSTAR machine and results analyzed using CellQuest software (Becton Dickinson).

To confirm the H2 genotype using probes for MHC Class II expression, PCR RFLP analysis was performed using the techniques of Peng and Craft specifically designed to differentiate $H2^k$ and $H2^b$ (37). Briefly, tail DNA was amplified using the primers IAAIF 5'GAAGACGACAT TGAGGCCGACCACGTAGGC 3' (SEQ ID NO:7) and IAAIR 5' ATTGGTAGCTGGGGTGGAATTTG ACCTCTT 3' (SEQ ID NO:8). PCR was performed with a 12 minute denaturation step of 94°C followed by cycles of 94°C for 20 sec, 60°C for 1 minute and 72°C for 1 minute with a final extension of 10 minutes at 72°C. The resultant PCR product was cut with Hind III and run in an agarose gel. Amplified DNA of the $H2^k$ genotype is cut while DNA of the $H2^b$ genotype is not allowing differentiation on agarose gel electrophoresis.

Urine protein excretion: Mice were placed in metabolic cages for 24 hour urine collection. To prevent bacterial growth, antibiotics (ampicillin, gentamicin, and chloramphenicol) were added to collection tubes. Urinary protein excretion was determined using the BioRad protein assay kit according to the manufacturer's instructions (BioRad, Hercules, CA) and reported as mg of protein/mouse/day.

Measurement of anti-DNA antibody: Anti-DNA levels were measured by ELISA as previously described (38). Briefly, 96 well ELISA plates were coated with 5μg/ml of double stranded calf thymus DNA (dsDNA) at 37°C overnight. The plates were then washed with PBS 0.05% tween (PBS-T). Sera were added in serial dilutions starting at a 1/100 dilution to each well, and incubated for 45 min at room temperature (RT). After washing with PBS-T, horse radish peroxidase (HRP) conjugated goat anti-mouse IgG (γ-chain specific, Sigma Chemical Co., St. Louis, MO) was added and incubated for 45 min. After additional washing, substrate solution containing 3, 3', 5, 5' tetramethylbenzidene (TMB, Sigma, St. Louis, MO) was added in 0.1M citrate buffer pH 4 and 0.015% H2O2. After incubation for 30 min, OD 380 absorption was determined on a Flow microtiter plate reader (Dynatech, Mclean, VA). Results are shown as the OD 380 absorbance at a 1/100 dilution. dsDNA was derived by S1 nuclease (Sigma, St. Louis, MO) treatment.

Isolation of cryoglobulins from sera: Cryoglobulins were isolated from sera as previously described (39). Briefly, blood samples were placed at 37°C for 2 hours. After centrifugation at 1000 rpm, supernatants were collected and placed at 4°C for 72 hours. They were then centrifuged at 3000 rpm, supernatants removed, and precipitates washed 5 times with cold PBS. After washing, they were resuspended in PBS at the same volume as the original sera. The isolated cryoglobulins were placed at 37°C for 3 hours before use.

Total Ig: Total IgG levels in sera or in cryoglobulins were determined by ELISA using a standard curve of known concentrations of mouse IgG. Cryoglobulins were placed at 37°C for 3 hours before use. ELISA plates were coated with 1μg/ml anti-

mouse κ overnight at 4°C. Sera or cryoglobulins were added in serial dilutions starting at a 1/1000 dilution. HRP conjugated goat anti-mouse IgG (γ-specific, Sigma, St. Louis, MO) was added, followed by TMB for color development. OD380 absorbance was measured as above. The same method was used for measurement of serum Ig isotype levels and total Ig in serum and in cryoglobulins except HRP goat anti-mouse IgG3, IgG1, or IgG2a, or HRP goat anti-mouse H & L chain were used (Southern Biotechnology, Birmingham, AL).

Measurement of circulating immune complexes: Circulating immune complexes were determined by the C1q ELISA method as previously described with minor modifications (40). ELISA plates were coated with human C1q in 0.1M Carbonate buffer (pH 9.6) and incubated for 48 hours at 4°C. After washing with PBS, PBS-1% BSA was added to each well and incubated for 2 hours at RT. EDTA treated sera samples (diluted 1/50) were added in duplicate, then incubated for 1h at RT and then overnight at 4°C. HRP anti-mouse IgG (γ chain specific, Sigma) was added in PBS-T. The rest of the assay was performed as described above. Aggregated human γ globulin was used as a positive control.

Rheumatoid factor: Rheumatoid factor (RF) levels in sera were measured by ELISA as previously described (33). ELISA plates were coated with rabbit IgG overnight at 4°C. After washing, sera were added in serial dilution starting at 1/100 dilution. The assay was then performed as described above using HRP conjugated goat anti-mouse IgG (γ chain specific, Sigma) or goat anti-mouse IgM (Sigma). For the measurement of IgG3 anti-IgG2a RF, the same method was used except ELISA plates were coated with mouse IgG2a and HRP anti-mouse IgG3 was used for detection of RF activity.

Measurement of C3 levels in sera: C3 levels in sera were measured by ELISA. 96 well ELISA plates were coated with goat anti-mouse C3 (Cappel, Durham, NC) and incubated overnight at 4°C. After washing and blocking with 5% BSA in PBS for 1-2h, samples diluted 1/200, 1/1000, 1/5000 and 1/25000 were added to individual wells, and

incubated for 1-2h at RT. After washing, rat anti-mouse C3 monoclonal antibody (RmC11H9) was added and incubated for 1h at RT (28). Peroxidase conjugated anti-rat IgG was added, then incubated for 1h at RT. 1mM ATBS in 0.1M citric acid buffer and 0.03% H2O2 was added for color development. The plates were read on an ELISA reader at OD 405.

Measurement of Bf levels in sera: Factor B levels were measured by ELISA. ELISA plates were coated with goat anti-human Factor B IgG fraction (DiaSorin, Stillwater, MN) overnight at 4°C. Sera diluted to 1/100, 1/200,and 1/400 were added to individual wells. Biotinylated anti-human Bf antibody (Diasorin) was added followed by avidin-peroxidase. TMB was added for color development. After incubation for 30 min, OD 380 absorption was determined.

Pathology: At the time of sacrifice (22 and 44 weeks), the kidneys were removed. One kidney was fixed with formaldehyde, embedded in paraffin, then sectioned before staining with hematoxylin and eosin (H&E) and PAS. The slides were read and interpreted in a blinded fashion grading the kidneys for glomerular inflammation, proliferation, crescent formation, and necrosis. Interstitial changes and vasculitis were also noted. Scores from 0-3 were assigned for each of these features and then added together to yield a final renal score (33). For example, glomerular inflammation was graded 0-normal, 1-few inflammatory cells, 2-moderate inflammation, 3-severe inflammation. The other kidney was snap frozen in liquid nitrogen and placed in OCT medium. 4um thick frozen sections were stained with fluorescein conjugated anti mouse IgG, IgM, IgA, IgG3 or C3 (Cappel, Durham, NC). Slides were read in a blinded fashion and graded 0-3+ (1+ mild, 2+ moderate, 3+ high staining).

Statistics: Statistical values were determined by using the Mann-Whitney two tailed U test or Kruskal-Wallis test. $p < 0.05$ was considered significant.

Phenotype of the mice: To determine the role of Bf in autoimmune nephritis, Bf knock out mice were backcrossed for 4 generations to MRL/lpr mice. The genotype of the mice was determined by PCR for Bf and fas. The percentage of offspring in the

litters derived were consistent with the predicted outcome, i.e. approximately 25% of pups were either homozygous knockout (B-/-, n=11) or wildtype (B+/+, n=14) while 50% were heterozygous for B- (n=20). Ten B+/- mice were randomly selected as the study mice. There were no differences in spleen weight or body weight between groups at 22 or 44 weeks of age (data not shown). By gross examination at the time of sacrifice, 100% of female B+/+ mice (7/7) had an excoriated skin rash on the shoulder area as well as tail and ear necrosis; 1/7 male B+/+ mice had ear and tail necrosis. Neither skin rash nor appendage necrosis was observed in B-/- or B+/- mice.

H2: To assess the MHC expression by each group, H2 expression of splenocytes was assessed by FITC staining and flow cytometry analysis. The MRL/lpr Bf deficient mice were $H2D^b$, the Bf heterozygotes $H2D^{b,k}$, and the wildtype mice were $H2D^k$. PCR RFLP analysis of MHC Class II expression confirmed the H2 haplotype of the mice with the B-/- mice being $H2^b$, B+/- mice were $H2^{b/k}$ and the B+/+ mice were $H2^k$.

Bf levels in sera: Bf levels in sera were measured by ELISA when the mice were 22 weeks of age. As shown in Table 1, no Bf was detected in the sera of B-/- mice confirming that the phenotype of the mice matched the genotype. Serum Bf levels of B+/+ mice were significantly higher than that of B+/- mice ($p \leq 0.05$) indicating a gene dose effect on serum Bf levels.

Urinary protein excretion: To assess the role of Bf in autoimmune nephritis, 24 hour urine protein excretion was measured beginning at 12 weeks of age. As shown in Figure 1, there was a statistically significant difference in proteinuria comparing B+/- mice after 20 weeks of age ($p \leq 0.05$) to both B+/+ mice and B-/- mice indicating the development of early severe disease in the heterozygous mice. The B+/+ mice developed increasing proteinuria after 26 weeks of age that was significantly greater than the B-/- mice ($p \leq 0.05$). Even at 44 weeks of age, B-/- mice still had not developed proteinuria >1 mg/mouse/day (data not shown).

Renal score: A group of mice were sacrificed at the age of 22 weeks (5 B-/-, 7 B+/+, and 3 B+/- mice) and pathologic renal scores were determined using a previously published scale for glomerular inflammation (33). The remaining B+/+ and B-/- mice

were sacrificed at 44 weeks (all B+/- mice died prior to 44 weeks). As shown in Figure 2A, Bf deficient mice had lower renal scores than the mice in the other groups at both 22 and 44 weeks. The renal abnormalities in the MRL/lpr B-/- mice consisted of focal glomerular hypercellularity without significant infiltrate of inflammatory cells (Figure 2B). Glomerular wire loop lesions, immune complex deposition, and glomerular hyalinization, as determined by intensity of PAS staining, were minimally present in the B-/- mice (Figure 2C). Renal findings in the MRL/lpr B+/- (data not shown) and B+/+ mice included diffuse glomerular hypercellularity, wire loop lesions, mesangial expansion, crescent formation, fibrinoid necrosis, glomerular hyalinization and infiltration of inflammatory cells (Figure 2D, 2E). The difference in renal scores in the B-/- compared to the other 2 groups at 22 weeks was not statistically significant ($p=0.13$) due to severe disease in one male B-/- mouse whose renal score was similar to that seen in the other two groups. The renal score of this mouse was included in the overall score and statistical analysis even though it exceeded the mean of the scores of the other B-/- mice by 6 standard deviations. To insure this mouse had been genotyped correctly, spleen DNA was amplified and results of the PCR amplification confirmed the B-/- genotype of the mouse. If the score for this mouse is excluded, the difference in renal score between the B-/- mice and either of the other two groups was significant at $p\leq 0.01$. The difference in renal scores between the B-/- mice and B+/+ mice was significant at 44 weeks ($p=0.04$).

Immunofluorescence analysis: To determine the effects of Bf genotypes on glomerular IC deposition, immunofluorescence analysis was performed. Frozen sections were stained with fluorescein conjugated anti-mouse C3, IgG, IgG3, IgM, and IgA. C3, IgM, and IgA deposition were similar in all groups with no apparent qualitative or quantitative differences (data not shown). Glomerular IgG3 deposition was minimal in all three groups. There was, however, significantly less IgG deposition in B-/- compared to B+/+ mice ($p\leq 0.05$, Table 2). IgG deposition in the kidneys of B-/- mice was also less than in the B+/- mice; the difference, however, did not reach statistical significance (Table 2).

Vasculitis: Medium to large vessel vasculitis is a pathologic feature of renal disease in MRL/lpr mice (41). At the age of 22 weeks, of the five B-/- mice examined, only the male B-/- mouse with severe renal disease had vasculitis (1/5). 50% of female B+/+ mice (2/4) had vasculitis as illustrated in Figure 2F, while no vasculitis was present in male B+/+ mice (0/3). In the B+/- mice, 1/3 of the mice had vasculitis; all three B+/- mice studied were females. At the age of 44 weeks, 100% of female B+/+ mice had vasculitis (3/3). There was no vasculitis in male B+/+ mice (0/4) nor vasculitis observed in the 6 B-/- mice (3 males and 3 females). These data indicate a greater incidence of vasculitis in the B+/+ mice than in the B-/- mice (overall 1/11 B-/- mice had vasculitis compared to 5/14 B+/+ mice). This difference in incidence of vasculitis was especially evident in female mice where 0/6 female B-/- mice had vasculitis compared to 5/7 female B+/+ mice ($p \leq 0.01$). The presence of renal vasculitis in the female B+/+ mice paralleled the vasculitis induced ear and tail necrosis present also primarily in the female B+/+ mice.

Measurement of serum anti-DNA antibody activity: To investigate possible mechanisms for the modulation of renal disease in the B-/- mice, levels of autoantibodies implicated in disease pathogenesis were measured. Anti-DNA antibodies are pathogenic in the autoimmune nephritis that develops in MRL/lpr mice (33). Anti-dsCT DNA antibodies in serum were measured by ELISA. As shown in Figure 3, serum anti-dsCT DNA antibody levels were lower at 16 weeks of age in B-/- mice compared to the other two groups ($p=0.02$). However, by 20 weeks and thereafter, the serum anti-DNA levels were not significantly different between the groups out to 44 weeks of age. Serum anti-glomerular binding activity, as measured by ELISA, paralleled anti-DNA activity with lower serum levels at 16 weeks in the B-/- mice that equilibrated between the groups over time (data not shown).

Measurement of RF: To assess the effect of Factor B on the production of other autoantibodies in the B-/- mice, serum RF activity was measured by ELISA. There were no detectable differences between the groups in production of total IgM RF or IgG RF (data not shown). However, as presented in Figure 4, significantly lower levels of IgG3 anti-IgG2a RF, a specificity linked with vasculitis in these mice, were present in the sera

of B-/- mice at the age of 20 weeks than in sera of the other 2 groups (41, 42). These differences were maintained through 44 weeks.

Immunoglobulin levels in sera or in cryoglobulins: MRL/lpr renal disease and vasculitis has been linked with IgG3 cryoglobulin production (41-44). To determine if the Bf genotype affected Ig or cryoglobulin production, total serum immunoglobulin levels and IgG3 cryoglobulin levels were measured by ELISA. Total IgG levels were similar at all time points in all 3 groups (data not shown), however, significantly higher levels of serum IgG3 and IgG3 cryoglobulins were present in the B+/- and B+/+ mice compared to the B-/- mice (Table 3, Figure 5). Serum levels of all other IgG isotypes were not statistically different between the 3 groups (data not shown).

C3 levels in sera: To determine the effects of a lack of Bf on serum C3 levels, C3 levels were determined by ELISA at the ages of 16, 20, and 24 weeks in all three groups. As shown in Table 4, serum C3 levels in the B-/- mice were equivalent to levels in normal C57BL6 mice and were significantly higher than serum C3 levels in the other two groups. Levels of serum C3 in the B-/- mice decreased over time suggesting some activation of C3, but were still significantly higher than the B+/+ mice even at 44 weeks of age (data not shown). These data indicate an unexpected significant role for Bf and the alternative pathway amplification loop in the consumption of C3 in this murine model of immune complex renal disease.

Circulating Immune Complexes: To assay for effects of Bf on clearance of circulating ICs, CIC were measured by ELISA using the C1q binding assay. There were no differences in sera CIC between groups at any time point (data not shown).

Treatment: To treat a subject with Factor B-mediated immune disease, the anti-Factor B antibody of the invention is administered parenterally, preferably intravenously, in a dosage determined in accordance with the method of the present invention. An effective dosage of the antibody is that dosage required to eliminate Factor B from the serum of the subject and to eliminate the activity of Factor B in the serum

In addition to monitoring serum Factor B levels, the subject's vital signs, complete blood count, volume of urine output and urinary protein excretion are routinely monitored to follow the progress of treatment. Specifically, the subject is monitored for blood pressure changes that may accompany kidney disease. One sign of efficacy of treatment is lowering of the subject's elevated blood pressure. Moreover, the subject's red blood cell count and hematocrit are monitored to determine whether the treatment is having a beneficial effect on erythropoiesis. Because subjects with complement-mediated glomerulonephritis are often anemic, a rise in the red blood cell count and hematocrit indicate the efficacy of treatment. Further, 24-hour urinary protein excretion is measured, and a decrease in protein excretion in the urine indicates efficacy of treatment. As kidney function improves, the subject's weight decreases as edema fluid is excreted.

Table 1. Sera fB levels in MRL/lpr mice

group (n)	fB levels in sera (OD 380)
B-/- (5)	0
B+/+ (7)	0.628 +- 0.04
B+/- (5)	0.444 +/- 0.04

Sera fB levels in B-/-, B+/+, and B+/- mice. Data presented are the mean OD 380 +/- standard error at 1/100 dilution of sera in each group at the age of 20 weeks. No fB was detected in sera of B-/- mice. fB levels in sera of B+/+ mice were significantly higher than that of B+/- mice (p=0.05 between B+/+ and B+/- mice).

Table 2. Immunohistology

group (n)	glomerular IgG deposition
B-/- (4)	0.88 +/- 0.13
B+/+ (7)	1.70 +/- 0.29*
B+/- (3)	1.33 +/- 0.70

Grade of immunofluorescence in B-/-, B+/+, and B+/- mice at 22 weeks of age. Data shown are the mean +/- standard error of the grade of immunofluorescence. Less IgG

deposition was observed in B-/- mice than in the other groups. *p<0.05 B+/+ mice versus B-/- mice.

Table 3. Sera IgG3 levels (mg/ml)

group (n)	sera IgG3 levels
B-/- (7)	0.9 +/- 0.32
B+/+ (9)	2.9 +/- 0.53*
B+/- (5)	4.9 +/- 1.25*
MRL/lpr (11)	4.2 +/- 0.81*

Sera IgG3 levels in B-/-, B+/+, B+/-, and MRL/lpr mice. Known concentrations of mouse IgG3 were used as a standard. Data shown are the mean IgG3 level (mg/ml) +/- standard error in each group at the age of 20 weeks. Sera IgG3 levels in B-/- were lower than the other groups. *p<0.05 versus B-/- mice.

Table 4. C3 levels (mg/ml) in sera of MRL/lpr mice.

group	16 weeks	20 weeks	24 weeks
B-/-	1.46 +/- 0.31 (n=5)	1.80 +/- 0.23 (n=5)	1.10 +/- 0.34 (n=6)
B+/+	0.60 +/- 0.09 (n=4)*	0.21 +/- 0.02 (n=7)*	0.08 +/- 0.05 (n=7)*
B+/-	1.00 +/- 0.14 (n=5)*	0.26 +/- 0.08 (n=6)*	0.48 +/- 0.07 (n=4)*
B6	1.54 +/- 0.18 (n=4)	ND	ND

Sera C3 levels in B-/-, B+/+, and B+/- mice. Data presented are the mean C3 level (mg/ml) +/- standard error. In B-/- mice, C3 levels were significantly higher than the other groups at all time points. *p<0.05 versus B-/- mice. ND: not determined.

Although the present process has been described with reference to specific details of certain embodiments thereof, it is not intended that such details should be regarded as limitations upon the scope of the invention except as and to the extent that they are included in the accompanying claims.

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

REFERENCES

1. Carroll, M. C. 1998. The role of complement and complement receptors in induction and regulation of immunity. *Annu. Rev. Immunol. 16:545.*

2. Muller-Eberhard, H. J., and R. D. Schreiber. 1980. Molecular biology and chemistry of the alternative pathway of complement. *Adv. Immunol. 29:1.*

3. Schreiber, R. D., M. K. Pangburn, P. H. Lesavre, and H. J. Muller-Eberhard. 1978. Initiation of the alternative pathway of complement: recognition of activators by bound C3b and assembly of the entire pathway from six isolated proteins. *Proc. Natl. Acad. Sci. USA 75:3948.*

4. Garnier, G., A. Circolo, and H. R. Colten. 1996. Constitutive expression of murine complement Factor B gene is regulated by the interaction of its upstream promoter with hepatocyte nuclear factor 4. *J. Biol. Chem. 271:30205.*

5. Holers, V. M. 1995. Complement. In *Clinical Immunology: Principles and Practice,* R. R. Rich, T. A. Fleisher, B. D. Schwartz, eds. Mosby, Inc., St. Louis, MO, p. 363.

6. Morris, K. M., D. P. Aden, B. B. Knowles, and H. R. Colten. 1982. Complement biosynthesis by the human hepatoma-derived cell line HepG2. *J. Clin. Invest. 70:906.*

7. Whaley, K. 1980. Biosynthesis of the complement components and the regulatory proteins of the alternative complement pathway by human peripheral blood monocytes. *J. Exp. Med. 151:501.*

8. Cole, F. S., E. E. Schneeberger, N. A. Lichtenberg, and H. R. Colten. 1982. Complement biosynthesis in human breast-milk macrophages and blood monocytes. *Immunol. 46:429.*

9. Katz, Y., F. S. Cole, and R. C. Strunk. 1988. Synergism between γ interferon and lipopolysaccharide for synthesis of Factor B, but not C2, in human fibroblasts. *J. Exp. Med. 167:1.*

10. Strunk, R. C., D. M. Eidlen, and R. J. Mason. 1988. Pulmonary alveolar type II epithelial cells synthesize and secrete proteins of the classical and alternative complement pathways. *J. Clin. Invest. 81:1419.*

11. Ripoche, J., J. A. Mitchell, A. Erdei, C. Madin, B. Moffatt, T. Mokoena, S. Gordon, and R. B. Sim. 1988. Interferon γ induces synthesis of complement alternative pathway proteins by human endothelial cells in culture. *J. Exp. Med. 168:1917.*

12. Praz, F. and E. Ruuth. 1986. Growth-supporting activity of fragment Ba of the human alternative complement pathway for activated murine B lymphocytes. *J. Exp. Med. 163:1349.*

13. Peter, M. G., J. L. Ambrus, A. S. Fauci, and E. J. Brown. 1988. The Bb fragment of complement Factor B acts as a B cell growth factor. *J. Exp. Med. 168:1225*

14. Hall, R. E., R. M. Blaese, A. E. Davis III, J. M. Decker, B. F. Tack, H. R. Colten, and A. V. Muchmore. 1982. Cooperative interaction of Factor B and other complement components with mononuclear cells in the antibody-independent lysis of xenogeneic erythrocytes. *J. Exp. Med. 156:834.*

15. Leijh, P. C. J., V. D. Barselaar, M. R. Daha, and R. V. Furth. 1981. Stimulation of the intracellular killing of staphylococcus aureus by monocytes: regulation by

immunoglobulin G and complement components C3/C3b and B/Bb. *J. Immunol. 129:332.*

16. Götze, O., C. Bianco, and Z. A. Cohn. 1979. The induction of macrophage spreading by Factor B of the properdin system. *J. Exp. Med. 149:372.*

17. Czop, J. and V. Nussenzweig. 1976. Studies on the mechanism of solubilization of immune precipitates by serum. *J. Exp. Med. 143:615.*

18. Takahashi, M., B. F. Tack, and V. Nussenzweig. 1977. Requirements for the solubilization of immune aggregates by complement: assembly of a Factor B-dependent C3-convertase on the immune complexes. *J. Exp. Med. 145:86.*

19. Takahashi, M., S. Takahashi, and S. Hirose. 1980. Solubilization of antigen-antibody complexes: a new function of complement as a regulator of immune reactions. *Prog. Allergy 27:134.*

20. Passwell, J., G. F. Schreiner, M. Nonaka, H. U. Beuscher, and H. R. Colten. 1988. Local extrahepatic expression of complement genes C3, Factor B, C2, and C4 is increased in murine lupus nephritis. *J. Clin. Invest. 82:1676.*

21. Manzi S., J. E. Rairie, A. B. Carpenter, R. H. Kelly, S. P. Jagarlapudi, S. M. Sereika, T. A. Medsger, and R. Ramsey-Goldman. 1996. Sensitivity and specificity of plasma and urine complement split products as indicators of lupus disease activity. *Arthritis Rheum. 39:1178.*

22. Carroll, M.C. 1998. The lupus paradox. *Nature Genetics 19:3.*

23. Prodeus, A. P., S. Georg, L-M. Shen, O. O. Pozdnyakova, L. Chu, E. M. Alicot, C. C. Goodnow, and M. C. Carroll. 1998. A critical role for complement in maintenance of self tolerance. *Immunity 8:721.*

24. Botto, M., C. Dell'Agnola, A. E. Bygrave, E. M. Thompson, H. T. Cook, F. Petry, M. Loos, P. P. Pandolfi, and M. J. Walport. 1998. Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nature Genetics 19:56.*

25. Fischer, M. B., M. Ma, S. Georg, X. Zhou, J. Xia, O. Finco, S. Han, G. Kelsoe, R. G. Howard, T. L. Rothstein, E. Kremmer, F. S. Rosen, and M. C. Carroll. 1996. Regulation of the B cell response to T-dependent antigens by classical pathway complement. *J. Immunol. 157:549.*

26. Korb, L. C. and J. M. Ahearn. 1997. C1q binds directly and specifically to surface blebs of apoptotic human keratinocytes: complement deficiency and systemic lupus erythematosus revisited. *J. Immunol. 158:4525.*

27. Walport, M. J., K. A. Davies, and M. Botto. 1998. C1q and systemic lupus erythematosus. *Immunobiol. 199:265.*

28. Quigg, R. J., A. Lim, M. Haas, J. J. Alexander, C. He, and M. C. Carroll. 1998. Immune complex glomerulonephritis in C4- and C3-deficient mice. *Kid. Int. 53:320.*

29. Sheerin, N. S., T. Springall, M. C. Carroll, B. Hartley, and S. H. Sacks. 1997. Protection against anti-glomerular basement membrane mediated nephritis in C3- and C-deficient mice. *Clin. Exp. Immunol. 110:403.*

30. Hebert, M-J, T. Takano, A. Papayianni, H. G. Rennke, A. Minto, D. J. Salant, M. C. Carroll, and H. R. Brady. 1998. Acute nephrotoxic serum nephritis in complement knockout mice: relative roles of the classical and alternative pathways in neutrophil recruitment and proteinuria. *Nephol. Dial. Transplant. 13:2799.*

31. Quigg, R.J., Y. Kozono, D. Berthiaume, A. Lim, D. J. Salant, A. Weinfeld, P. Griffin, E. Kremmer, and V. M. Holers. 1998. Blockade of antibody-induced glomerulonephritis with Crry-Ig, a soluble murine complement inhibitor. *J. Immunol. 160:4553.*

32. Quigg, R.J., C. He, A. Lin, D. Berthiaume, J. J. Alexander, D. Kraus, and V. M. Holers. 1998. Transgenic mice overexpressing the complement inhibitor crry as a soluble protein are protected from antibody-induced glomerular injury. J. Exp. Med. *188*:1321.

33. Watson, M. L., J. K. Rao, G. S. Gilkeson, P. Ruiz, E. M. Eicher, D. S. Pisetsky, A. Matsuzawa, J. M. Rochelle, and M. F. Seldin. 1992. Genetic analysis of MRL-lpr mice: relationship of the Fas apoptosis gene to disease manifestations and renal disease-modifying loci. *J. Exp. Med. 176:1645.*

34. Vidal, S., D. H. Kono, and A. N. Theofilopoulos. 1998. Loci predisposing to autoimmunity in MRL-Fas^{lpr} and C57BL/6-Fas^{lpr} mice. *J. Clin. Invest. 101:696.*

35. Vyse, T. J. and B. L. Kotzin. 1998. Genetic susceptibility to systemic lupus erythematosus. *Annu. Rev. Immunol. 16:261.*

36. Matsumoto. M., W. Fukuda, A. Circolo, J. Goellner, J. Strauss-Schoenberger, X. Wang, S. Fujita, T. Hidvegi, D. D. Chaplin, and H. R. Colten. 1997. Abrogation of the alternative complement pathway by targeted deletion of murine Factor B. *Proc. Natl. Acad. Sci. USA 94:8720.*

37. Peng S.L. and J. Craft. 1996. PCR-RFLP genotyping of murine MHC haplotypes. *Biotechniques. 21:362*

38. Gilkeson, G. S., J. Grudier, D. Karounos, and D. Pisetsky. 1989. Induction of anti-dsDNA antibodies in normal mice by immunization with bacterial DNA. *J. Immunol. 142:1482.*

39. Abdelmoula, M., F. Spertini, T. Shibata, Y. Gyotoku, S. Luzuy, P-H. Lanbert, and S. Izui. 1989. IgG3 is the major source of cryoglobulins in mice. *J. Immunol. 143:526.*

40. Toth, C. A., D. Pohl, and Y. Agnello. 1986. Methods for detection of immune complexes by utilizing C1q or rheumatoid factors. In *Manual of Clinical Laboratory Immunology*, N. R. Rose, H. Friedman, and J. L. Fahey, eds. American Society for Microbiology, Washington, D. C., p. 204.

41. Nose, M., M. Nishimura, M. R. Ito, J. Toh, T. Shibata, and T. Sugisaki. 1996. Arteritis in a novel congenic strain of mice derived from MRL/lpr lupus mice; genetic dissociation from glomerulonephritis and limited autoantibody production. *Am. J. Pathol. 149:1763.*

42. Fulpius, T., F. Spertini, L. Reininger, and S. Izui. 1993. Immunoglobulin heavy chain constant region determines the pathogenicity and the antigen-binding activity of rheumatoid factor. *Proc. Natl. Acad. Sci. USA 90:2345.*

43. Lemoine, R., T. Berney, T. Shibata, T. Fulpius, Y. Gyotoku, H. Shimada, S. Sawada, and S. Izui. 1992. Induction of "wire-loop" lesions by murine monoclonal IgG3 cryoglobulins. *Kid. Int. 41:65.*

44. Fulpius, T., R. Lemoine, T. Berney, Y. Pastore, S. Moll, and S. Izui. 1996. Polymorphonuclear leukocytes play a key role in the generation of "wire-loop" lesions induced by a murine IgG3 rheumatoid factor. *Kid. Int. 49:647.*

45. Chan O. T. M., L. G. Hannum, A.M. Haberman, M.P. Madaio, and M.J. Shlomchik. 1999. A novel mouse with B cells but lacking serum antibody reveals an antibody-independent role for B cells in murine lupus. *J. Exp. Med. 189:1639.*

46. Boackle, S. A., M. A. Morris, V. M. Holers, and D. R. Karp. 1998. Complement opsonization is required for presentation of immune complexes by resting peripheral blood B cells. *J. Immunol. 161:6537.*

47. Boackle, S. A., V.M. Holers, and D.K. Karp. 1997. CD21 augments antigen presentation in immune individuals. *Eur. J. Immunol. 27:122..*

48. Gilkeson, G. S., G. Garnier, A. Circolo, V. M. Holers, A. Feagin, R. Wetsel, H. Colton. 1998. Modulation of renal disease in lupus mice deficient in complement component C3. *Mol. Immunol. 35:36. (abstract)*

49. Schwaeble, W., Luttig, B., Sokolowski, T., Estaller, C., Weiss, E., Buschenfelde, K., Whaley, K., and Dippold, W. 1993. Human Complement Factor B: Functional Properties of a Recombinant Zymogen of the Alternative Activation Pathway Convertase. *Immunobiol., 188: 221-232.*

What is claimed is:

1. A method of treating or preventing Factor B-mediated immune disease in a subject, comprising administering to the subject an inhibitor of synthesis or activity of Factor B, whereby the inhibition of synthesis or activity of Factor B treats or prevents the immune disease.

2. The method of claim 1, wherein the complement-mediated immune disease is selected from a group consisting of vasculitis, systemic lupus erythematosus, rheumatoid arthritis, myocardial infarction, ischemic/ reperfusion injury, cerebrovascular accident, Alzheimer's disease, transplantation rejection (xeno and allo), all antibody-mediated skin diseases, all antibody-mediated organ-specific diseases (including Type I and Type II diabetes mellitus, thyroiditis, idiopathic thrombocytopenic purpura and hemolytic anemia, and neuropathies), multiple sclerosis, cardiopulmonary bypass injury, membranoproliferative glomerulonephritis, polyarteritis nodosa, Henoch Schonlein purpura, serum sickness, Goodpasture's disease, systemic necrotizing vasculitis, post streptococcal glomerulonephritis, idiopathic pulmonary fibrosis (usual interstitial pneumonitis) and membranous glomerulonephritis.

3. The method of claim 1, wherein the subject is a mammal.

4. The method of claim 1, wherein the subject is a human.

5. The method of claim 1, comprising contacting the subject with an antibody to Factor B, under conditions that permit the binding of the antibody to Factor B in the subject, whereby the binding of the antibody to Factor B reduces the serum level or activity of Factor B, thereby reducing formation and deposition of immune complexes in the subject.

6. The method of claim 5, wherein the antibody is monoclonal that blocks Factor B activity.

7. The method of claim 5, wherein the antibody is purified polyclonal.

8. A composition comprising an anti-Factor B antibody and a pharmaceutically acceptable carrier.

9. The method of claim 1, wherein the inhibitor of synthesis or activity of Factor B is a nucleic acid that encodes an anti-sense oligonucleotide which blocks the synthesis of Factor B, whereby blocking the synthesis of Factor B in the subject reduces the serum level and activity of Factor B, thereby reducing formation and deposition of immune complexes in the subject.

10. An anti-sense oligonucleotide capable of hybridizing to the mRNA that encodes Factor B.

11. A vector comprising the oligonucleotide of claim 10.

12. A composition comprising the vector of claim 11 and a pharmaceutically acceptable carrier.

13. The method of claim 1, wherein the inhibitor of synthesis or activity of Factor B is a peptide that mimics the binding properties of Factor B for Factor D but lacks the ability to activate C3, whereby the binding of the peptide to C3 does not activate C3 and blocks the effects of Factor B on C3, thereby reducing formation and deposition of immune complexes in the subject.

14. A peptide that mimics the binding properties of Factor B but lacks the ability to activate C3, whereby the binding of the peptide to C3 does not activate C3 and blocks the effects of Factor B on C3, thereby reducing formation and deposition of immune complexes in the subject.

15. A composition comprising the peptide of claim 14 and a pharmaceutically acceptable carrier.

16. A method of diagnosing a subject as having a Factor B-mediated immune disease, comprising measuring the serum level and activity of Factor B in the subject or determining the amount of complement activation in the subject, whereby an elevated serum level of Factor B, elevated activity of Factor B or increased complement activation in the subject diagnose the subject as having a Factor B-mediated immune disease.

17. A method of determining the efficacy of a treatment in a subject with Factor B-mediated immune disease, comprising measuring the serum level and activity of Factor B and determining the amount of complement activation, before and after the treatment, the lower the level of complement activation, the more effective the treatment.

18. A transgenic mouse, comprising a mutation in the Fas gene and a mutation in the gene producing Factor B.

19. The mouse of claim 18, wherein the mutant is the MRL-lpr Factor B knockout mouse.

20. A method of determining the efficacy of treatment in and prognosis of a subject with Factor B-mediated immune disease, comprising measuring the serum level of C3, whereby a lower than normal serum level of C3 indicates a poor prognosis and lack of efficacy of treatment, and whereby a rising serum level of C3 from a lower than normal level to a nearly normal level of C3 indicates efficacy of treatment and an improving prognosis.

Figure 1

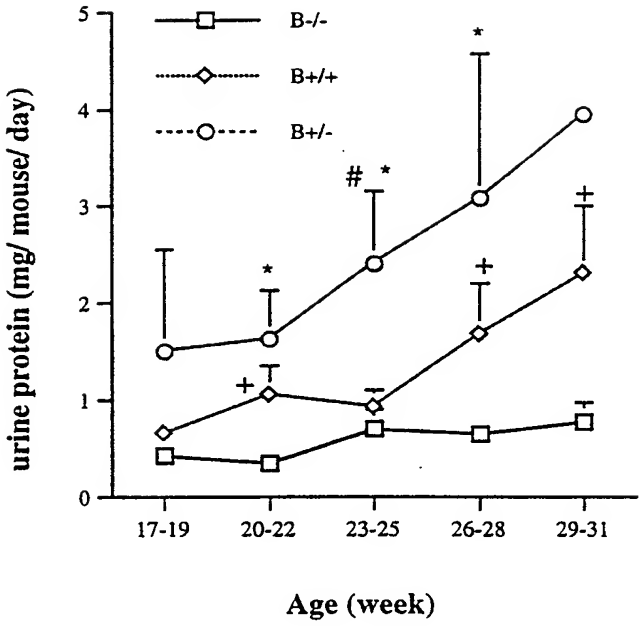

B-/-
B+/+
B+/-
urine protein (mg/ mouse/ day)
0
1
2
3
4
5
17-19
20-22
23-25
26-28
29-31
Age (week)
*
#
+

Figure 2A

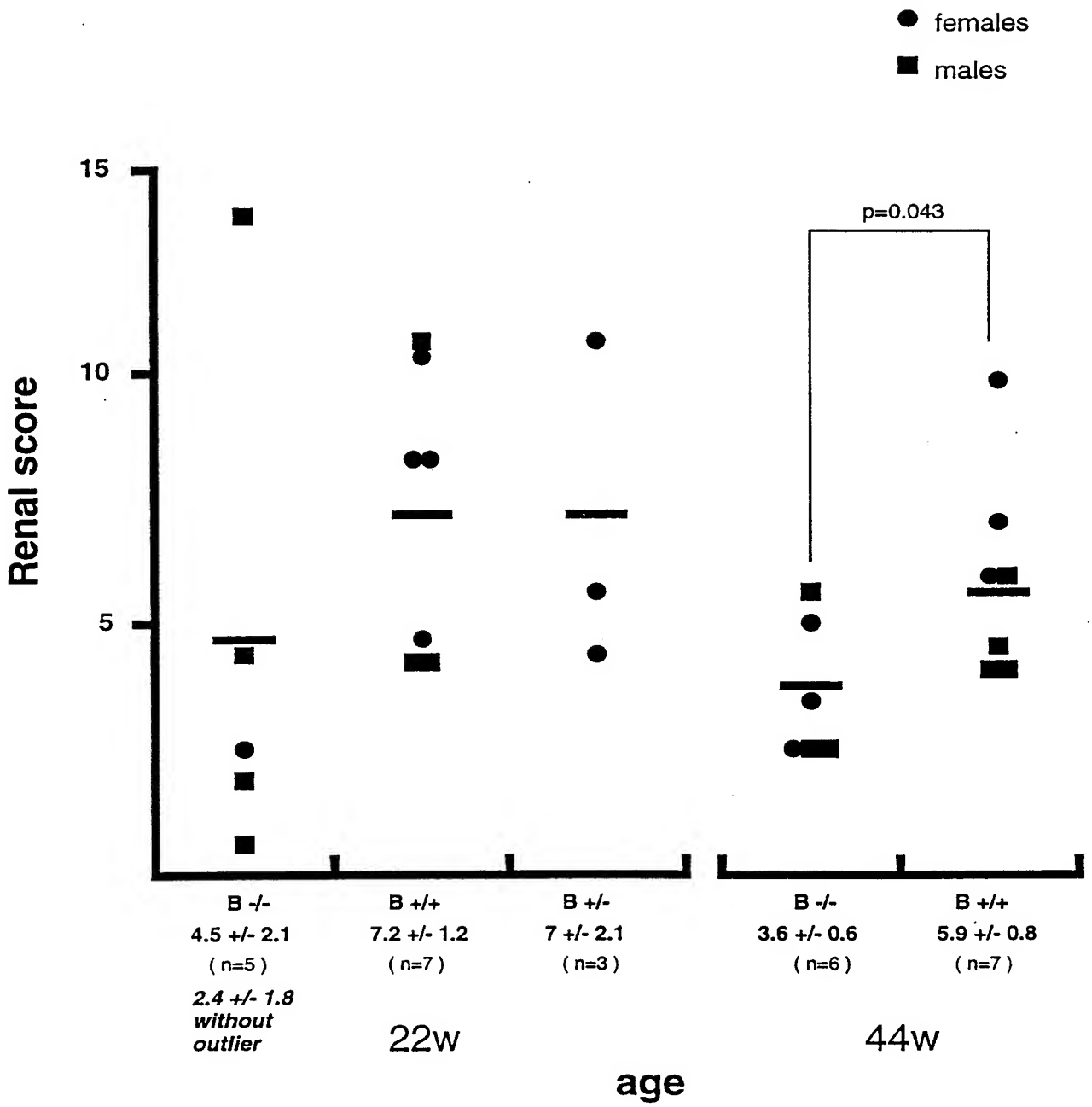

females
males
15
10
5
Renal score
p=0.043
B -/-
4.5 +/- 2.1
(n=5)
2.4 +/- 1.8
without
outlier
B +/+
7.2 +/- 1.2
(n=7)
B +/-
7 +/- 2.1
(n=3)
22w
B -/-
3.6 +/- 0.6
(n=6)
B +/+
5.9 +/- 0.8
(n=7)
44w
age

Figure 3

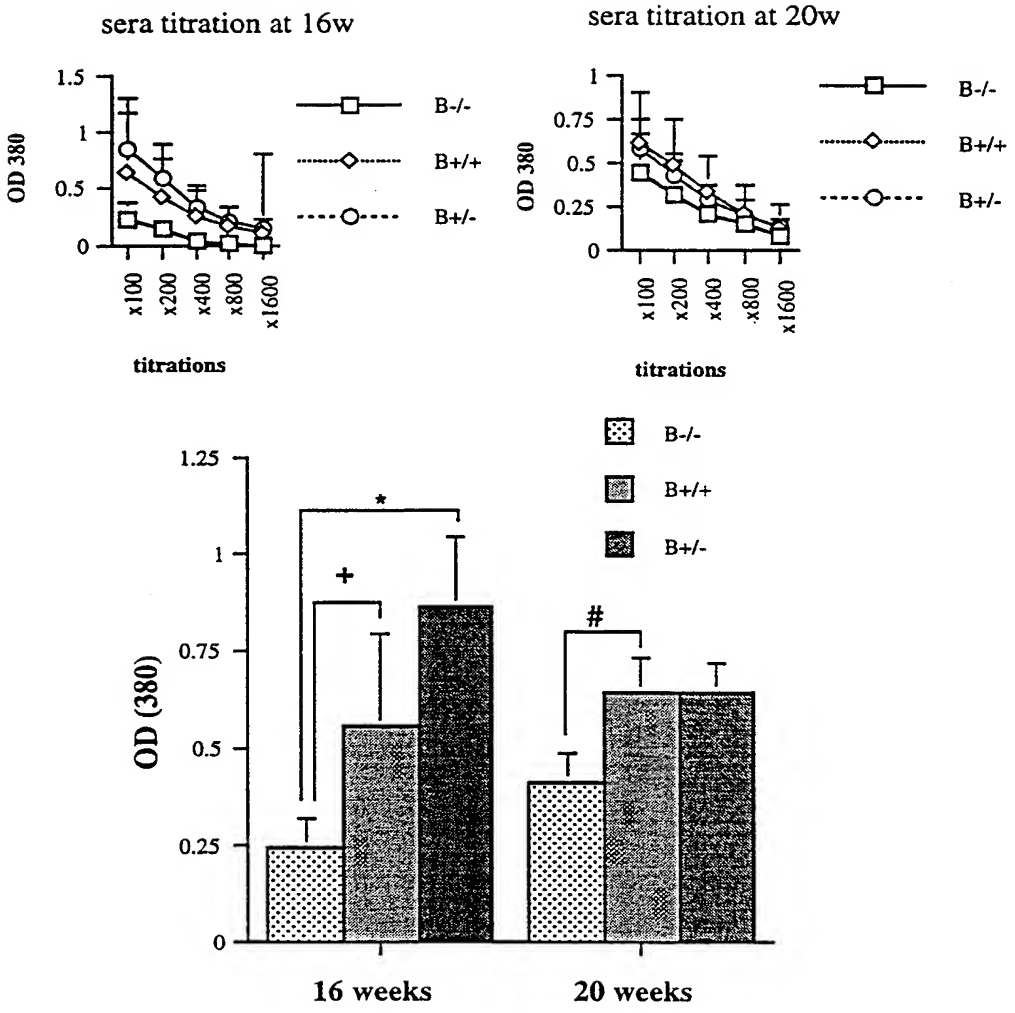

sera titration at 16w
OD 380
1.5
1
0.5
0
x100
x200
x400
x800
x1600
titrations
B-/-
B+/+
B+/-
sera titration at 20w
OD 380
1
0.75
0.5
0.25
0
x100
x200
x400
x800
x1600
titrations
B-/-
B+/+
B+/-
OD (380)
1.25
1
0.75
0.5
0.25
0
*
+
#
B-/-
B+/+
B+/-
16 weeks
20 weeks

Figure 4

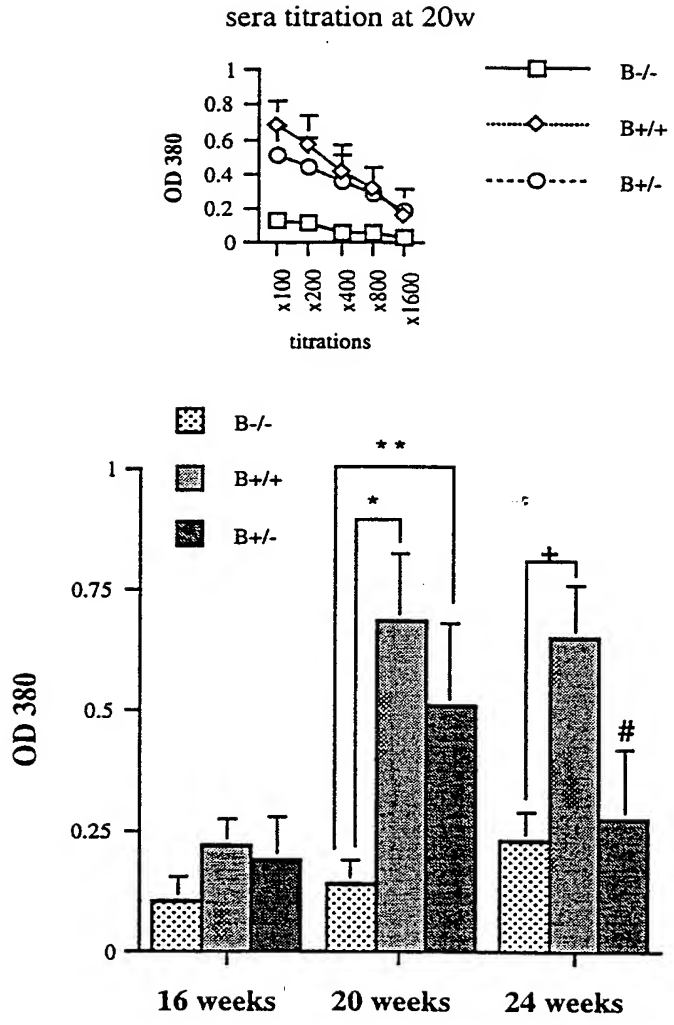

sera titration at 20w
OD 380
1
0.8
0.6
0.4
0.2
0
x100
x200
x400
x800
x1600
titrations
B-/-
B+/+
B+/-
B-/-
B+/+
B+/-
OD 380
1
0.75
0.5
0.25
0
*
**
+
#
16 weeks
20 weeks
24 weeks

Figure 5

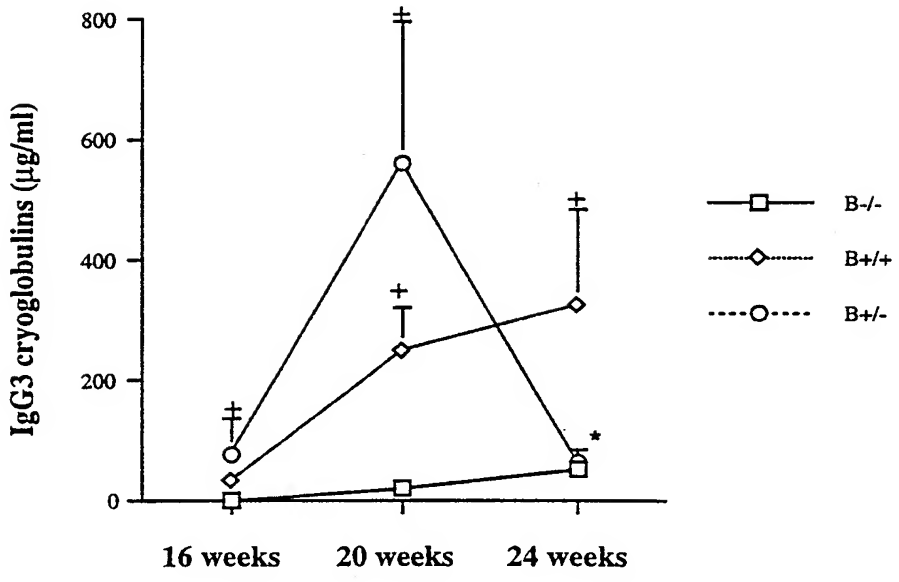
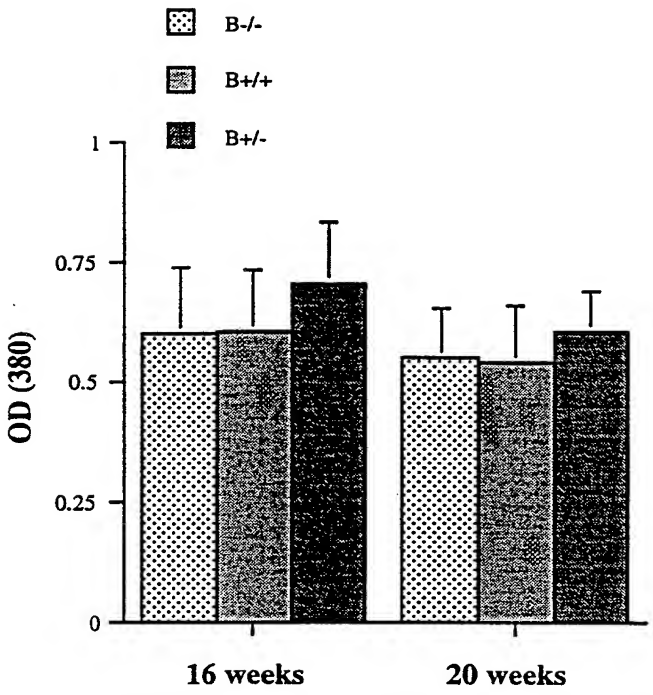

800
600
400
200
0
IgG3 cryoglobulins (µg/ml)
16 weeks
20 weeks
24 weeks
*
B-/-
B+/+
B+/-

Figure 6



SEQUENCE LISTING

<110> MUSC Foundation for Research Development

GARY S. GILKESON
HIROSHI WATANABE

<120> BLOCKING FACTOR B TO TREAT COMPLEMENT-MEDIATED IMMUNE DISEASE

<130> 19113.0074/P

<150> 60/103,660
<151> 1998-10-09

<160> 8

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223>Description of Artificial Sequence:/Note =
synthetic construct

<400> 1
ccgaagcatt cctatcctcc 20

<210> 2
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223>Description of Artificial Sequence:/Note =
synthetic construct

<400> 2
cgaatgggtg accgcttcc 19

<210> 3
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223>Description of Artificial Sequence:/Note =
synthetic construct

<400> 3
gtagtcttgt ctgctttctc c 21

<210> 4
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223>Description of Artificial Sequence:/Note =
synthetic construct

<400> 4
aggttacaaa aggtcaccc 19

<210> 5
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223>Description of Artificial Sequence:/Note =
synthetic construct

<400> 5
gatacgaaga tcctttcctg tg 22

<210> 6
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223>Description of Artificial Sequence:/Note =
synthetic construct

<400> 6
caaacgcagt caaatctgct c 21

<210> 7
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223>Description of Artificial Sequence:/Note =
synthetic construct

<400> 7
gaagacgaca ttgaggccga ccacgtaggc 30

<210> 8
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223>Description of Artificial Sequence:/Note =
synthetic construct

<400> 8
attggtagct ggggtggaat ttgacctctt 30